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BIODYNAMICS OF MICROECOSYSTEMS

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UNPUBLISHED PRELIMINARY DATA

This report covers the period between July 23, 1964 and January 23, 1965. The first six months of this grant were spent mainly setting up the experimental equipment and in the initial phases of the work. Most of the required items of equipment were purchased and delivered to either the Savannah River Plant (SRP) or the University of Georgia at Athens. Only two major pieces of equipment remain undelivered. These are two walk-in constant temperature and humidity rooms. At present, they are being tested by the manufacturer and will be shipped within two weeks. Following the installation of the two rooms, the facilities at the SRP will be complete. The necessary routine procedures for microecosystem research have been worked out and a technician trained to perform them.

Although insufficient space for the culture of microecosystems existed during the first six month period, a few experiments were carried out and limited data were obtained. A summary of the four catagories of work started, along with preliminary results, are given below.

1. Studies with a Simplified Microecosystem Derived From a Sewage Oxidation Pond

The first work carried out under this grant concerned itself with a microecosystem originally isolated from a sewage oxidation pond in Austin, Texas in 1959. It is one of the several types of self-contained and regenerative microecosystems simplified from various natural ecosystems that are scheduled for study during the next two years. The biological components of this system are two algae (*Chlorella* and *Schizothrix*), several protozoa, an ostrocod (*Cypridopsis*), 15 to 20 bacteria, and two rotifers. The system will live well on a completely defined medium. This medium consists of half strength Taub #36 medium (Taub and Dollar, 1964) plus amino acids or peptones. The system has survived as a regenerative system in the laboratory for several years with light as the only energy imput.

When a representative sample of the microecosystem (about 2.5 ml) is inoculate into sterile, fresh medium (usually 125 ml in a 250 ml erlenmyer flask) a heterotrophic succession takes place. This succession is defined as heterotrophic because the biomass is first increased by the growth of the heterotrophic organisms such as bacteria living on the peptones or amino acids. After the bacterial bloom, there is an algal bloom and the system begins to function autotrophically with plants providing the fixed carbon and energy necessary for the other members of the community. After the algal bloom, the system settles into a steady-state situation which persists indefinitely. The advantages of this microecosystem as an experimental tool are (1) its ease of handling, (2) its reproducibility, and (3) its relatively short period of succession. This period is measured in days as opposed to the succession found in most ecosystems, which is measured in months or years. One of the primary objectives of the research under contract nsG-706/11-003-001 is to test the hypothesis that true stability in a life support system is obtainable only after the system becomes adjusted to boundary condition (i.e., outside environment) by the process of ecological succession. Thus, the study of "microsuccession" is a first order of research under this contract.

Experiments with Heterotrophic Succession. Several different experiments are being conducted to study quantitative aspects of the heterotrophic succession. The time from inoculation to algal bloom has been found to vary with temperature. This period is approximately five days at 30°C and 30 days at 10°C. The changes in biomass during succession are also being investigated. A 1 to 5 mg. inoculum in a 125 ml microecosystem will increase to about 22 mg at bloom time. Maximum biomass obtainable under optimum condition is about 60 mg per microcosm or 0.48 g per liter. All these figures are expressed as dry weight. Some functions of the organic substrate have been investigated. Most of the experiments use 0.5 grams per liter mixed peptones (Fisher multi-peptone or Difco protease-peptone) as part of the starting medium. We are now trying to determine the minimum amounts of these or other organics which are required for a normal heterotrophic succession. Although it has been shown that certain amino acids can be substituted for the mixed peptones in the heterotrophic succession, the work is being repeated under more favorable conditions in an attempt to quantify the results.

Experiments with Autotrophic Succession. The simplified microecosystem will not grow after being inoculated into half strength Taub solution without added organic matter. Although the Taub solution is an algal growth medium it appears that at half strength it has insufficient nitrogen to permit any appreciable autotrophic increase in the biomass of the system. In an autotrophic succession the biomass must increase through an initial growth of the green plants. The increase in green plant biomass is followed by (i.e. makes possible) the growth of heterotrophic organisms feeding on the green plants or on products derived from them.

If nitrate nitrogen is added in an amount equal to that present in the peptone heterotrophic medium, then autotrophic succession occurs. Since the *Chlorella* requires thiamine, this vitamin must also be added to the medium. Autotrophic succession is both slower and ultimately results in lower final biomass figures than heterotrophic succession. At present an attempt is being made to increase the speed and amount of growth through the use of reduced (ammoniacal) rather than oxidized (nitrate) nitrogen.

2. Nutritional Experiments

A recent publication (Myers, 1964) suggests that the introduction of other organisms into the food chain of a human life support system should be investigated. As a step in this direction, animal feeding experiments with the dried biomass of the simplified ecosystem are to be undertaken. A unique opportunity presents itself at the SRP Ecology Laboratory. Dr. Frank Golley, a leading ecological bioenergetici is available for consultation and assistance. He will cooperate in this phase of the work. Pilot experiments have shown that it is feasible to mass culture the microecosystem in five gallon batches. Each batch yields a little over 7 g dry weight of material. This material will be pelletized and fed to known breeding pairs of house mice. Other pairs will be fed a standard laboratory ration as a control. Changes in weight and litter production will be used as criteria in judging the adequacy of dried microecosystem as a food. Since it has already been shown that food from pure algal cultures in an inadequate diet for most mammals, it will be interesting to see if the yield from a complete ecosystem, with its greater diversity of organic material, will provide a more adequate diet for mammals.

3. Studies with Temporary Pond Microecosystems

Temporary pond microecosystems are of special interest because of their tolerance of extreme condition of hydration and dehydration. Samples of dried sediment have been collected from stations located in an arc around the Gulf of Mexico from Palm Beach, Florida to Padre Island, Texas. Equal weights of these sediments (250 g.) have been placed in 2.5 liter jars under grow-lux fluorescent light bulbs. The lights are controlled by a timer to produce a twelve hour photoperiod. The jars were initially filled with distilled water. More water was added in sufficient quantities to permit a slow reduction of the total amount of water in each jar over a two month period. Almost all of the water has now evaporated. The systems will be left dry for another two months and then the wet cycle repeated. At that time metabolic measurements of the systems will be made following the method of Beyers, 1963.

4. Graduate Training in Microecosystem Ecology

One of the primary objectives of the grant is to assist in training of students in the metabolism of ecosystems, a new field of great interest and importance both from the standpoint of space travel and from the standpoint of man's continued survival in his present "macroecosystem" — the biosphere.

Accordingly, a set up for student research and training is being constructed on the campus at Athens so that students may gain experience before undertaking thesis research with the more elaborate equipment at SRP. One Ph. D. student, Mr. Richard Gingrich, has already begun training and will undertake thesis research next year. Mr. Gingrich is testing the Margalef (1963) hypothesis that plant pigment ratios indicate the stage in succession of ecosystems, and, therefore, relative stability of the system. Since pH has already been found to be a useful variable in monitoring the relationship between production (photosynthesis) and respiration in microecosystems, we hope that easily measured pigment ratios may prove useful in monitoring biochemical diversity and stability in the system.

Applicants are now being screened for assignment to the program for the coming academic year (see attached announcement).

Literature Cited

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